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6. AUTHOR(S)
E. M. Goodman, B, Greenebaum

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Biomedical Research Institute
University of Wisconsin-Parkside
900 Wood Road
Kenosha WI 53141

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
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13. ABSTRACT (Maximum 200 words)
We have previously shown that exposing *Escherichia coli* to a magnetic fields (MFs) increased the levels of some proteins and decreased others. Using a cell-free system we reported that expression (transcription and/or translation) of the α and $\beta\beta$' subunits of DNA-directed RNA polymerase were elevated following exposure to 45 Hz, 1.1, 0.21 and 0.07 mT magnetic fields.

In the last phase of this program, we sought to determine whether transcription and/or translation were altered by MFs. A cell-free *E. coli* system was used to determine whether or not MFs directly affect the translational or translational processes. Our data suggest that MFs have no significant direct effect on either transcription or translation. In contrast, when a eukaryotic *HeLa* extract containing a *cmyc* plasmid was exposed to a magnetic field, enhanced transcription was observed. At this time we conclude that our inability to distinguish differences in transcription and translation in *E. coli* may be related to the coupled nature of these processes in the bacterial system.

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OBJECTIVE: We have shown that exposing *Escherichia coli* to a magnetic field (MF) increased the levels of some proteins and decreased others (Goodman et al, 1994a). Using a cell-free system we reported that expression (transcription and/or translation) of the α and $\beta\beta'$ subunits of DNA-directed RNA polymerase were elevated following exposure to 45 Hz, 1.1, 0.21 and 0.07 mT magnetic fields (Goodman et al, 1994b). In the last phase of this program, we sought to determine whether transcription and/or translation were altered by MFs.

A cell-free *E. coli* system was used to determine whether or not MFs directly affect the translational process. Our data suggest that MFs have no significant direct effect on translation. In addition, we also attempted to show that transcription was altered directly by MF-exposure in this model system; no significant differences between control and exposed cultures were observed. However, development of more precise assays on transcription and translation with cell-free systems might show significant differences.

MATERIALS and METHODS

MF-Effects on Translation in a Cell-Free System: Plasmid PM47 (gyrase b) obtained from Dr. Martin Gellert (Laboratory of Molecular Biology, N.I.H.) was isolated and purified by CsCl centrifugation using standard protocols. The cell-free expression system was prepared using *E. coli* BL-21 obtained from Dr. Richard Burgess, McArdle Laboratory for Cancer Research (Madison, WI, USA). Preparation of the extracts and the experimental procedures have been described previously (Goodman et al, 1994b).

Briefly, cell-free experiments to examine MF-effects on translation were performed by preparing a single tube reaction mix (400 μl) containing 165 μl Tris EDTA buffer (pH, 8.0), 165 μl S-30 extract, nucleotides (40 mM ATP, 10 mM CTP, 10 mM UTP 10 mM GTP), 1.0 mM cAMP, 20 μg/ml folinic acid, 1 mM dithiothreitol, 1 mg/ml t-RNA, 19 amino acids (all at 0.5 mM, no methionine), 0.5 μl of [^{35} S] methionine (spec. act. 47.5 TBq/mM), and salts containing optimized concentrations of Mg^{2+}, Ca^{2+} and NH_4 ions. Supercoiled, CsCl-purified gyrase b (*gyr b*)-containing plasmids (100 pg) were added to 400 μl of an S-30 reaction mix. The reaction mix was pre-incubated in a 37°C water bath for 5 minutes; two 25 μl samples were removed and served as ice blanks. Following removal of the blanks, rifampicin (20 μl of rifampicin (100 mg/ml)) was added to the mix. Rifampicin binds to the β subunit of RNA polymerase, inhibiting the initiation of new mRNA's to inhibit further mRNA transcription. The mix was then split into two tubes; one of the tubes was placed in the control incubator (no applied field) and the other was placed in the MF-exposure incubator (see field exposure below). Samples (25 μl) were withdrawn from each tube at 5, 10, 15 and 20 minutes intervals and pipetted to 25 mm nitrocellulose filters (0.45 μ); the order of sampling was determined using a random number table. A few drops of 1N NaOH was added immediately to remove the ^{35}S labelled methionine from unincorporated tRNA's; 25% cold trichloroacetic acid (TCA) was then added to the filters. The filters were placed in scintillation vials and washed three times with 5% TCA with vigorous shaking; after rinsing in 95% ethanol, the filters were dried in a Speed Vac™ and counted in a scintillation counter.

MF-Effects on Transcription in a Cell-Free System: To insure that transcription of the plasmid was occurring the S-30 reaction mix was prepared as described above with the following modifications. Transcription was analyzed using denaturing gel electrophoretic techniques. The 19 amino acid solution used in the expression system described above was replaced with a mix containing 20 amino acids (@ 0.5 mM). The nucleotide uridine triphosphate (UTP) was replaced by [α-^{32}P] UTP. For these experiments the reaction tubes were placed in the control and MF-exposure incubators for 5 minutes; the reaction was stopped by adding a solution containing 6 M ammonium acetate, 20 mM EDTA and 1 mg/ml tRNA). The RNA was precipitated with 100 μl of absolute ethanol by centrifugation at 10,000 x g. RNA was resuspended in 10 μl of loading buffer (80 % formamide, 0.1% xylene cyanol, 0.1 % bromphenol blue, 2 mM EDTA) and heated at 90 °C for two minutes. Samples were resolved on a prewarmed (50 °C) 0.75 mm thick, 8 M urea 8% polyacrylamide gel using the Bio-Rad II minigel apparatus™ at 200 Volts for one hour.

Field Exposure: Duplicate EMF exposure coils were placed in two separate incubators (Warren-Sherer RL-8) set at 36.6°C; incubators were shut off during an experiment. The exposure coils consisted of 40 turns of #16 copper magnet wire wrapped around the outside of a 500 ml cylindrical water jacketed vessel (LG8012, Lab Glass, Vineland, NJ). The coils generate a vertical magnetic field. Both water jacketed vessels were connected to a circulating Haake A80 water bath (Saddlebrook, NJ) maintained at 36.6°C. The diameter of the coils is approximately 15 cm; exposed and control cultures were contained in a 10 mm x 75 mm polystyrene tubes centered in the midplane of the coils inside the water-jacketed vessels. The induced electric field pattern for this configuration is a series of concentric circular field lines; the intensity is zero at the center of the tube and rises linearly with radius to a maximum of approximately 10^{-4} V/m at the edge of the tube.

The coil in the experimental incubator was excited in series by a Techcron Model 7541 power amplifier, which is amplifying a signal generated by a Wavetek Model 125 arbitrary waveform generator. Prior to initiating an experiment the temperature in sample flasks placed in the exposure and control positions were monitored using a Flüke 52 K/J thermocouple thermometer (Everett, WA).

Statistical Analysis: The control and experimental data from each experiment were fit to a linear regression line. The slopes as a function of exposure time were then compared using a paired-t test ($p < 0.05$ = significance). The data from the total expression system only examined protein synthesized after 20 minutes. These data were analyzed using the non parametric Wilcoxon signed ranks test.

RESULTS and DISCUSSION

The *Escherichia coli* cell-free transcription/translation systems has been used in our lab because it offers a more controlled experimental system for experiments designed to understand how electromagnetic fields might induce bio-effects. In the experiments described above, we examined the direct effect of magnetic fields (MFs) on translation of gyrase b (*gyrb*). Translation of *gyrb* was examined in an S-30 expression system in which the transcription of new mRNA was inhibited after a defined period of time. Translation kinetics in a MF and

control environment were followed by measuring the rate of ^{35}S methionine incorporation into protein. Samples placed in control and MF-exposure environments were withdrawn and handled in a random manner.

To insure that equivalent mRNA was present in both the control and MF-exposed systems, the S-30 system containing plasmid was incubated for 5 minutes in a common reaction mix. Samples were also removed to examine the effects of MFs on total expression. At the end of the pre-incubation period, rifampicin was added to prevent the synthesis of any new mRNA. At this point, the reaction mix was divided into two tubes, one was placed in a control environment and the other was placed in the MF-exposure system and translation was allowed to proceed for periods ranging from 1 to 20 minutes. Samples were withdrawn counted and their regression lines computed as described. A comparison of the slopes of control and MF-exposed extracts summarized in Table 1 show no statistically significant difference in ^{35}S methionine labelled proteins when the starting level of mRNA is equivalent. In contrast, experiments using a cell-free expression coupled transcription/translation system in the presence of a magnetic field showed elevated *gyrb* expression following a 20 minute exposure (Table 2). The later data are consistent with earlier studies on α and β polymerase that also showed elevated expression under similar conditions (Goodman *et al.* 1994b). Based on these collective data we conclude that weak MFs do not enhance or affect translation directly.

To determine that transcription of the plasmid was occurring in the cell-free system, a reaction mix containing a labelled nucleotide ([α-^{32}P] ATP) was analyzed using denaturing gel electrophoretic techniques. Autoradiograms (data not shown) indicate that *gyrb* was being transcribed. We also attempted to determine whether or not enhanced transcription could be detected in a cell-free S-30 system exposed to MFs. In these experiments transcription was examined at 1,3 5 and 7 minutes and the results analyzed by fitting to data to a regression line and comparing slopes. No statistically significant difference was evident when the slopes were compared (Table 3).

Based on differences obtained from our total expression system and other published reports on enhanced mRNA synthesis in the presence of an applied field, an experiment was undertaken to ascertain whether or not the bacterial expression system was capable of resolving small differences in mRNA levels. The question might be restated as to whether or not the coupled nature of the bacterial transcription/translation system may be a mitigating factor in our experimental design. To address this question we exposed a *HeLa* nuclear extract containing a *cmyc* plasmid to a similar MF environment. The data shown in Table 4 indicate that mRNA transcription is significantly enhanced in a nuclear extract exposed to MF's.

These *E. coli* data thus present the anomalous situation where the individual processes transcription or translation do not show statistically significant differences when extracts are exposed to MFs, whereas the coupled system does show a statistically significant difference. In the context of the present experiment, Collectively, we interpret these data to show that in the coupled system, a small but not statistically significant amount of mRNA is produced in the presence of a magnetic field. Because transcription and translation are coupled in bacteria, small transcriptional differences could be amplified during the translation process

ultimately producing a significant difference. This interpretation is consistent with the data reported here which show that when the mRNAs are equivalent at the initiation of translation, no amplification of the mRNA signal occurs in the presence of a magnetic field.

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Goodman, E.M., Greenebaum, B., and Marron, M.T. (1994b) Altered protein synthesis in a cell-free system exposed to a sinusoidal magnetic field. Biochim. Biophys. Acta 1202, 107-112.

Goodman, E.M., Greenebaum, B., and Marron, M.T. (1994) Transcription and translation in a cell-free system exposed to a sinusoidal magnetic field. 16th Ann. Meeting Bioelectromagnetics Soc. p55.

ACCOMPLISHMENTS: The cell-free expression experiments containing a *gyr b* plasmid showed enhanced expression of mRNA following 1, 3 and 5 minute exposure to a 45 Hz 1.1 mT magnetic field. No differences were seen at 7 minutes. The absence of differences at 7 minutes may be due to the action of RNAses in the S-30 system. In contrast, MF-exposure did not enhance the protein synthesis over a 20 minute experimental period.

SIGNIFICANCE: The plasma membrane has often been invoked as a primary site of interaction between weak electromagnetic fields and cells. The data from cell-free expression experiments unequivocally show that an intact membrane is not a requirement to induce an EMF bioeffect. The data show that MFs enhance mRNA transcription in a eukaryotic but not a procaryotic cell-free system. The translation data indicate that this process is not directly altered by field-exposure; however, development of more precise assays on transcription and translation with cell-free systems might show significant differences. Collectively, these data indicate that the use of cell-free systems may present a unique and novel approach for elucidating the mechanism of interaction between weak EMF's and living systems

PUBLICATIONS AND ABSTRACTS (last 12 months):

1. Goodman, E.M., Greenebaum, B., and Marron, M.T. (1995) Effects of electromagnetic fields on molecules and cells. Int. Rev. Cytology. 158, 279-338.

2. Greenebaum, B., Goodman, E.M., Marron, M.T. (1994) Transcription and translation in a cell-free system exposed to a sinusoidal magnetic field. 14th Ann Meeting Society for Physical Regulation in Biology and Medicine.

Table 1.

A comparison of linear regression slopes from translation experiments (N=8) in which *E. coli* cell-free system containing the *gyrb* plasmid were exposed to 45 Hz 1.1 mT. In these experiments transcription has been inhibited by addition of rifampicin after a 5 minute incubation period. A paired-t analyses of the slopes indicate the data are not significantly different ($P = 0.24$)

Control	MF-exposed
4094 ± 1510*	5099 ± 1269

* mean ± standard error of the mean

Table 2.

A comparison of expression (transcription and translation in an *E. Coli* S-30 cell-free system containing the *gyrb* plasmid exposed to 45 Hz, 1.1 mT fields (N=8) over a 20 minute period. A Wilcoxon Signed Rank Analyses of the non-parametric data indicate the data are significantly different (P = 0.02)

Control	MF-exposed
174,880 ± 46,882*	277,010 ± 71,476

* mean ± standard error of the mean

Table 3.

A comparison of linear regression slopes from transcription experiments (N = 20) in which *E. coli* cell-free extracts containing the *gyrb* plasmid were exposed to 45 Hz, 1.1 mT fields. Transcription was examined at defined intervals during a 7 minute incubation period. A paired-t analyses of the slopes show that the data are not significantly different (P = 0.9)

Control	MF-exposed
3247 ± 559*	3222 ± 550

* mean ± standard error of the mean

Table 4.

The effecy of 60 Hz, 1.1 mT MF expoure on transcription in a *HeLa* nuclear extract conyaining the *cmyc* plasmid.

Control	MF-exposed
$1.06 \pm .26x10^{5*}$	$1.65 \pm .65x10^{5}$

* mean counts (^{32} P-UTP incorporation) ± standard error of the mean; the difference is significant ($p=0.02$).